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Research Article

Festuca silana (Poaceae), a new species from the Sila plateau in Italy

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Abstract

A new hexaploid species of fine-leaved fescue from *Festuca* sect. *Festuca* (*Festuca silana* Ardenghi, Pallanza & Foggi, **sp. nov.**) endemic to the Sila plateau is described. The new taxon shows morphological affinities with species of the *F. marginata* group from which it can be distinguished by higher ploidy, ecology, and leaf cross section anatomy. ddRADseq data suggests *F. silana* could be a local hexaploid descendant/derivate of the widespread diploid *F. marginata*. Its distribution is restricted to several localities in the Sila highlands of the Southern Apennines, Italy.

Key words: Calabria, endemic, fescue, flow cytometry, Italy, morphometry, new species, ploidy, RAD sequencing

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Introduction

Festuca L. (Poaceae, Loliinae) is one of the most species-rich genera of grasses (Poaceae), containing worldwide around 680 accepted species (Govaerts 2024). Within the *Festuca* genus, "fine-leaved fescues" represent the most important group, with approximately 450 species (Foggi and Tison 2014; Kellogg 2015). The taxonomy and systematics of *Festuca* has historically been regarded by botanists as difficult, due to the morphological convergence of unrelated taxa, phenotypic plasticity, complex nomenclature and a lack of clear universally accepted diagnostic features between taxa. The first solid foundation to modern taxonomic *Festuca* studies was posed by Eduard Hackel in his "*Monographia Festucarum Europaearum*" (Hackel 1882). Hackel introduced novelties in the approach to identification and classification of fescues, including the study of the leaf cross-section, one of the main investigation tools still in use in modern festucology. Since then, many scholars have dedicated their efforts toward the taxonomy and systematics of *Festuca*, integrating the initial morphometrical approach with more modern techniques stemming from technologicals and scientific advances. These include analysis of the relative

genome size (Šmarda et al. 2008; Martínez-Sagarra et al. 2021), chromosome number (Šmarda and Kočí 2003; Ardenghi et al. 2016), DNA sequencing (Boeuf et al. 2022, Kriuchkova et al. 2023, Mucko et al. 2024) and electron microscopy (Ortúñez and Cano-Ruiz 2013). Although the implementations of these new methods emanate from improved floristic records and checklist (Roleček et al. 2019; Gudkova et al. 2023; Bednarska et al. 2024), to the conservation and applications of the taxa studied (Ardenghi et al. 2017), most recent works have focused on groups of species, using an integrated approach combining multivariate morphometry, ploidy level and genetics (Šmarda et al. 2007; Foggi et al. 2012; Ardenghi et al. 2024; Bednarska et al. 2024).

During an extensive sampling conducted across the European Mediterranean basin in 2014 by N.M.G. Ardenghi, an unusual taxon belonging to the fine-leaved fescues was collected in different localities of the Sila plateau (Italy). The entity was already known and reported by Sarfatti in the "Prodoromo della Flora della Sila" under *Festuca ovina* L. subsp. *laevis* Hack. var. *gallica* (Hack.) St.-Yves subvar. *costei* St.-Yves (≡ *Festuca costei* (St.-Yves) Markgr.-Dann.) based on the original identification by Markgraf-Dannenberg (Sarfatti 1959). However, this taxon has been overlooked until now. A preliminary study of morphology and leaf cross section anatomy of both fresh samples and herbaria specimens including the holotype for *F. costei*, led us to question the identification proposed by Markgraf-Dannenberg. In fact, these fescues differ from *F. costei* in a series of readily identifiable features, especially in the leaf cross-section (e.g. number of vascular bundles, development and organization of the sclerenchyma, etc.) and rather appear to be morphologically in between the *F. stricta* and the *F. marginata* groups. In particular, the Sila entity appears to have the typical cross sections of the former and the overall morphology of the latter.

Due to the unusual combination of leaf cross-section anatomy and general morphology, we initially thought it could be a taxon of the *F. stricta* group (i.e.: *F. stricta* Host, *F. rupicola* Heuff., *F. trachyphylla* (Hack.) R.P.Murray) which present similar patterns in the organization of the tiller leaves sclerenchyma. This is especially true for *F. trachyphylla*, which is also the only species from the stricta group to normally present 7 vascular bundles in the cross section, a shared characteristic with the Sila specimens. Nevertheless, the samples still share numerous morphological similarities (e.g. smooth leaf blades, glabrous spikelets, etc.) with *Festuca marginata* (Hack.) K. Richt., which occurs in similar types of habitats across Italy.

To better understand the relationships among these different taxa, we chose to apply an integrated approach combining classical morphometry, ploidy level analysis using flow cytometry and ddRADseq sequencing. The results from these combined analyses support that the taxon from the Sila plateau is a new endemic species.

Materials and methods

Materials

Overall, 177 individuals representing 25 populations of five different taxa (*F. marginata*, *F. rupicola*, *F. stricta*, *F. trachyphylla* and the proposed new species) ranging from the Alps to the Apennines and from fresh and herbarium

material (BRNU, FI, G, MSNM, PAV; Herbaria codes follow Thiers 2024, updated continuously) were morphologically studied (Suppl. material 1: tables S4, S5). Specimens were carefully selected to ensure a balanced representation of the different taxa. Fifty of the freshly collected individuals from the same populations were also analyzed using flow cytometry.

Morphometric analysis

We selected 35 morphological characters for the analysis (Table 1), choosing those considered as diagnostic for fine-leaved fescues.

Measurements were performed according to the standards described in Foggi 1999 (which complies with Hackel 1882; Saint-Yves 1913; Ellis 1976; Wilkinson and Stace 1991) with minor modifications. To account for intraindividual variation, we chose to either keep the highest recorded value for the sample or to use the mean value calculated from three different measurements depending on the morphological character. Characters concerning leaf cross sections follow a design similar to Bednarska et al. (2024). Spikelet, floret and other microscopic characters were observed under a Eurotek NB-50T stereomicroscope at magnifications of 8x–10x. Leaf cross sections were studied with a Carl Zeiss Axiostar Plus microscope at magnifications of 40x–100x. Both microscopes were coupled with a camera (ToupTek USB3.0 Eyepiece Camera S3CMOS05000KPA) and all observed characters were measured using Toupview ver. 4.11.19728.20211022 software.

Each quantitative character was tested for normality of distribution within taxa using the Shapiro-Wilk test. Some characters had non-normal distribution for some taxa, however, since the chosen analyses have been shown to be robust to violation of the normality of distribution assumption (Klecka 1980), we decided to continue without transforming the data. To further support this decision, it has to be clear that non-fitted variables would have to be transformed as a whole, independently from the taxon, leading to a weakened perception of the actual morphological variability among taxa. Characters were also tested for significant correlations (>0.95) via Spearman's non-parametric coefficient. High correlation was found only between characters related to the tiller leaves' scabridity (Sk_Deg, Sk_Ext, Ep_Ind). Among them, we decided to keep only the density of the abaxial epidermis indumentum as observed in the cross section (Ep_Ind) in the analyses as it was the least subjective to measure. We also decided not to include the culm length and tiller leaf blade length (Clm_L and LfB_L respectively) due to their high dependency on environmental factors such as grazing, trampling and wildfires. However, these characters were systematically measured in all samples and used in the morphological description of the new species. After preliminary data manipulation, 31 of the original characters were used to perform the analyses.

A PCoA utilizing Gower's distances (Gower 1971) was used to visualize the pattern of morphological variation among the studied taxa. Following the PCoA, a jackknifed canonical discriminant analysis (CDA) was performed (Krzanowski 1990), taking into account the morphological groups individuated with the PCoA and the ploidy levels inferred with flow cytometry. All characters invariant within the groups were excluded from the CDA dataset (bringing down the characters used to 25) as it is one of the fundamental assumptions to avoid any type of distortion in Discriminant Analyses.

Table 1. Morphological characters used in multivariatue analyses and their coding. QD = quantitative discrete, QC = quantitative continuous, BI = binary, CO = ordinal.

Code	Character	Type
Clm_L	Culm length	QC
LfB_L	Tiller leaf blade length	QC
Pan_L	Panicle length	QC
Pan_Sk	Panicle scabridity	CO
Pan_Pb	Pubescence at the base of the panicle	CO
Clf_L	Culm leaf blade length	QC
Sh_Nd	Sheath to node distance	QC
InfBr_L	Inferior branch of the panicle length	QC
TilSh_Pb	Tiller sheaths pubescence	CO
Nd_Pr	Node pruinosity	BI
Sh_Pr	Tiller sheaths pruinosity	BI
Sh_Col	Tiller sheaths color	CO
Sk_Deg	Tiller leaves scabridity degree	CO
Sk_Ext	Percentage of tiller leaves scabrid surface	QC
Sc_Org	Sclerenchyma organization	CO
Sc_CT	Central sclerenchyma strand thickness	CO
Sc_MT	Marginal sclerenchyma strands thickness	CO
KlMR	Keel to middle rib distance	QC
Klmg	Keel to margins distance	QC
VB_N	Number of vascular bundles	QD
R_N	Number of accessory ribs	QD
Shp	Leaf cross-section outline shape	CO
Ep_Und	Presence of epidermal undulations	BI
Ep_Ind	Richness of epidermal indumentum	CO
LH	Longest hair in the abaxial surface of the cross-section	QC
Sp_L	Spikelet length	QC
LGl_L	Lower glume length	QC
UGl_L	Upper glume length	QC
UGl_hW	Upper glume half-width	QC
Lm_L	Lemma length	QC
Lm_hW	Lemma half width	QC
A_L	Awn length	QC
Gl_Pb	Glumes pubescence	CO
Lm_Pb	Lemma pubescence	CO
Sp_Sh	Spikelet shininess	CO

All statistical analyses were computed with R (R Core Team 2024) in RStudio 2024.4.2.764 (Posit team 2024) using the MorphoTools2 package (Šlenker et al. 2022).

Ploidy estimation

The ploidy level was measured in 50 samples (1–4 per population) using flow cytometry with DAPI dye. The youngest and most well-preserved leaves were selected from representative individuals of both fresh plants and herbarium vouchers (no older than one year). Samples were then co-chopped with the standard (*Lycopersicum esculentum* "Stupické polní tyčkové rané") in a Petri dish containing 0.5 mL Otto I buffer (0.1M citric acid, 0.5% Tween 20; Otto

1990) using a razor blade. The nuclei suspension was then filtered through a 50 µm nylon mesh before 1 mL of Otto II buffer (0.4M Na2HPO4 · 12H2O) supplemented with 2 µg/mL DAPI was added. Samples were analyzed with a CyFlow ML flow cytometer (Partec GmbH, Germany) equipped with a UV light-emitting diode (365 nm, Sysmex Partec GmbH) at the Department of Botany and Zoology, Masaryk University, Brno, Czech Republic. To confirm the ploidy level, choromosome counts were performed using the Fuelgen protocol. In brief, Squash preparations were made on root tips obtained from germinating seeds. The root tips were pre-treated with 0.4% colchicine for 3 hours and then fixed in Carnoy fixative solution for 1 hour. After hydrolysis in HCl 1N at 60 °C for 7–8 minutes, the tips were stained in leuco-basic fuchsine for 3 hours.

DNA extraction and ddRAD sequencing

Genomic DNA was extracted from silica gel-dried leaves or herbarium specimens from 14 samples of the same species included in the morphometric analyses (Suppl. material 1: table S7). DNA quality was assessed by 1.5% agarose gel electrophoresis, and concentration was measured using a Qubit 2 Fluorometer with the 1X dsDNA HS Assay Kit (Thermo Fisher Scientific).

The double digest restriction site-associated DNA (ddRAD) library preparation protocol was adapted from Sochor et al. (2024) with modifications. Briefly, 100 ng of genomic DNA was digested with SbfI-HF and MseI restriction enzymes in rCutSmart buffer (New England Biolabs) at 37 °C for 3 hours, followed by enzyme inactivation at 80 °C for 20 minutes. Immediately afterwards, P1 and P2 adapters, corresponding to the restriction sites of the respective enzymes, were ligated using T4 DNA ligase (New England Biolabs) at 16 °C overnight, with subsequent heat inactivation at 65 °C for 10 minutes. The P1 adapter for each sample contained a unique barcode for individual identification. The DNA concentration of each restricted sample was measured using the Qubit fluorometer, and equimolar amounts of digested DNA from all samples were pooled. The pooled samples were purified with 1.2 × SPRI magnetic beads and used as a template for PCR enrichment. PCR amplification was performed in four 20 µl reactions (18 cycles each) using Phusion HF PCR Mastermix (New England Biolabs) and standard Illumina P1-i5 (5’-AATGATACGGCGACCACCGA-3’) and P2-i7 (5’-CAAGCAGAAGACGGCATACGA-3’) primers. The protocol for each step is available in the Suppl. material 1: table S6. The final amplified products were size-selected using SPRI magnetic beads, with left and right side selections at 0.5 × and 0.9 × ratios, following the manufacturer’s protocol. The final ddRAD library was sequenced at the CEITEC facility (Brno, Czech Republic) on an Illumina NextSeq platform using a mid-output configuration with 300 cycles. Sequencing utilized a portion of the platform’s capacity, generating approximately 30,000,000 paired-end reads.

Phylogenomic analysis

Paired-end reads generated by Illumina sequencing of the ddRAD library were demultiplexed and analyzed using iPyRAD v.0.9.97 (Eaton and Overcast 2020). Quality control parameters were configured as follows: trimming was performed for bases with a quality score below Q20, allowing up to five low-quality bases per read. The Phred Q score offset was set to 33. The minimum read depth for

base calling and majority-rule consensus was set to 6, with a maximum read depth per sample capped at 10,000. The sequence similarity threshold was specified as 0.90, permitting a maximum of one base mismatch in barcodes. Adapter sequences were strictly filtered out, and a minimum read length of 35 bp was required. Consensus sequence assembly was conducted with the following parameters: a maximum of six alleles per consensus sequence to accommodate diploid and polyploid species in the dataset; a maximum of 5% uncalled bases and 5% heterozygous sites per consensus sequence; and a minimum of seven samples sharing data at a given locus (allowing up to 46% missing data). Locus filtering parameters were set to allow a maximum of 20% SNPs per locus, up to eight indels per locus, and a maximum of 20% heterozygous sites per locus.

Phylogenomic relationships among individuals based on ddRAD data were inferred using RAxML v.8.2.12 (Stamatakis 2014). The GTRCAT substitution model was employed, and bootstrap analysis with 1,000 replicates was performed to construct a maximum likelihood tree. The resulting phylogeny was visualized using FigTree v.1.4.4 (Rambaut 2015).

Results

Morphometric analysis

The first two axes of the exploratory PCoA account for 45.23% of the variability. Three main clusters can be observed in the biplot (Fig. 1): species of *F. stricta* group, the Sila hexaploid and the diploid *F. marginata*. The hexaploid taxon from the Sila plateau appears morphologically as an in-between entity but still clearly distinct from the other two groups. In particular, the taxon seems to share with the species of the *F. stricta* group the tendency to develop decurrent to confluent sclerenchyma strands (Sc_Org), as well as presenting undulations between epidermal cells (Ep_Pap) and longer awns (A_L). However, these are the only similarities between the two groups, as the Sila specimens resemble more, on a superficial level, species of the *F. marginata* group. They lack numerous other features typical

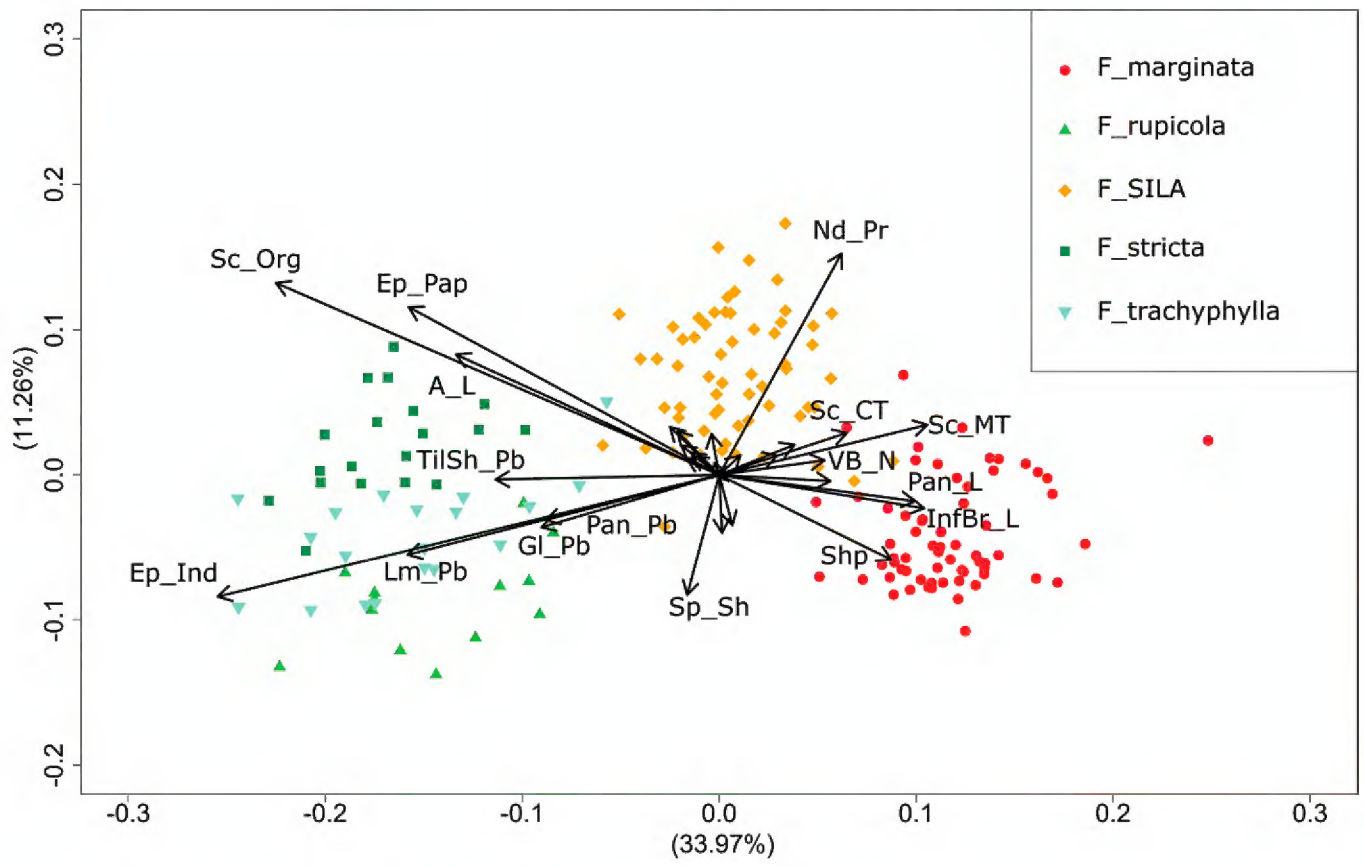

Figure 1. Biplot for the PCoA performed with Gower's distance.

of the *F. stricta* taxa, such as scabrid leaf blades due to the presence of barbs on the adaxial indumentum (Ep_Ind) and an overall pubescence observed in the panicle (Lm_Pb, Gl_PB, Pan_Pb) and tiller sheats (TilSh_Pb). Compared to the new species, *F. marginata* tends to develop discrete thickened sclerenchyma strands (Sc_Org, Sc_MT, Sc_CT), a larger number of vascular bundles (VB_N), bigger panicles(Pan_L, InfBr_L) and leaves in a more elongated conduplicate "V" shape (Shp). Finally, the Sila taxon seems to be overall a more pruinous plant (Nd_Pr, Sp_Sh) compared to all the other species analyzed. Based on the clustering of the taxa in the biplot, we decided to merge the species from the *Festuca stricta* aggregate into a single group to avoid distortions in the following discriminant analysis.

The jackknifed CDA (Fig. 2) fully supports the grouping hypothesis indicated by the scatterplot of the PCoA with 97.74% classification success in 177 samples (Table 2). Again, the new species appears as a distinct morphological in-between entity of the other considered species.

Table 2. Confusion matrix for the DA performed on the three morphological groups individuated by the PCoA.

Taxon	N	*F. marginata*	*F. sila*	*F. stricta* group	correct	%
F. marginata	65	65	0	0	65	100.00
F. silana	61	3	58	0	58	95.08
F. stricta group	51	0	1	50	50	98.04
Total	177	68	59	50	173	97.74

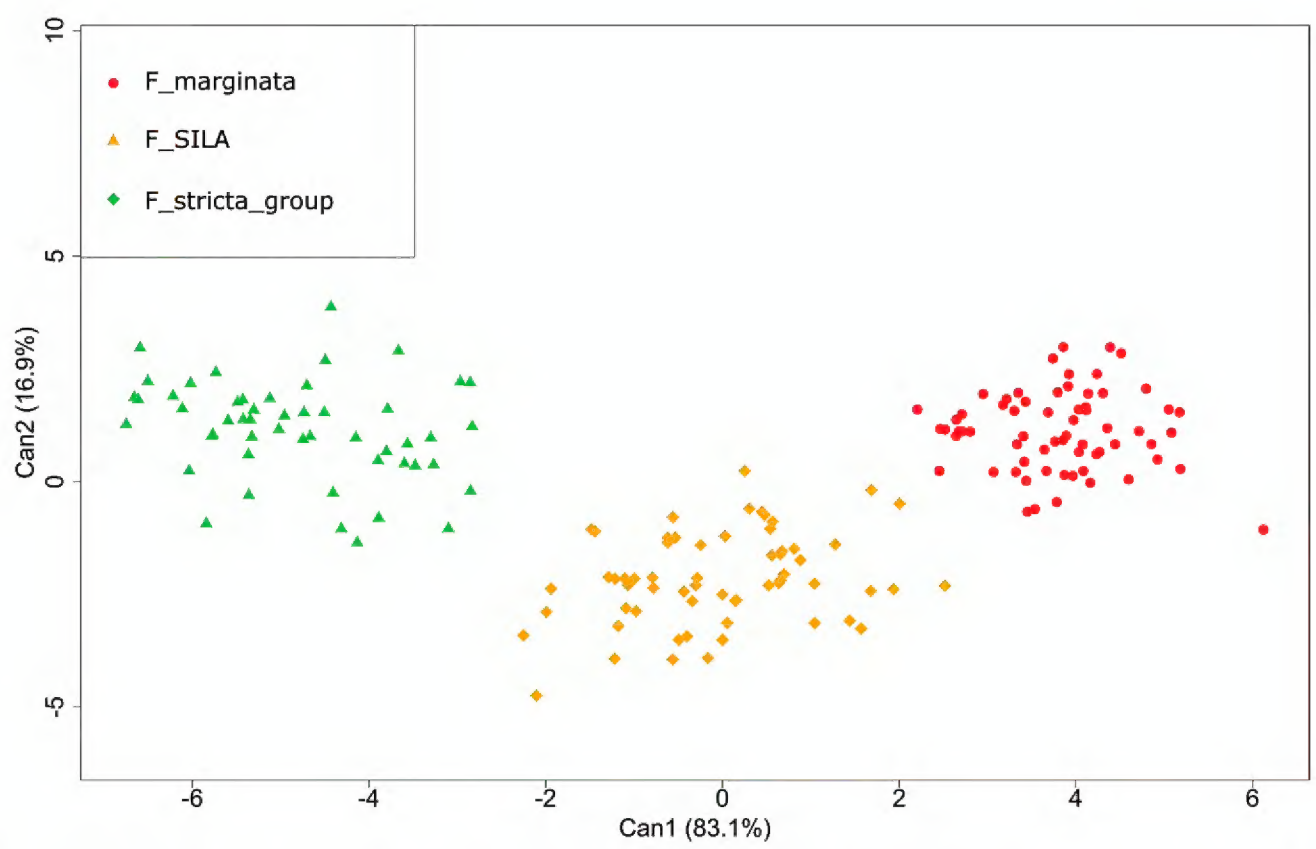

Figure 2. Scatterplot for the DA performed on the three morphological groups individuated by the PCoA.

Ploidy estimation

Overall, 50 of the freshly collected individuals were studied with flow cytometry. All 20 individuals of *F. stricta* s.l. (*F. stricta*, *F. trachyphylla* and *F. rupicola*) were hexaploid and all *F. marginata* samples were diploid (17 individuals) in accordance with previous works (Portal 1999; Arndt 2008, Šmarda et al. 2008; Ardenghi et al. 2016, 2024). All 13 individuals assumed as *F. silana* were hexaploid.

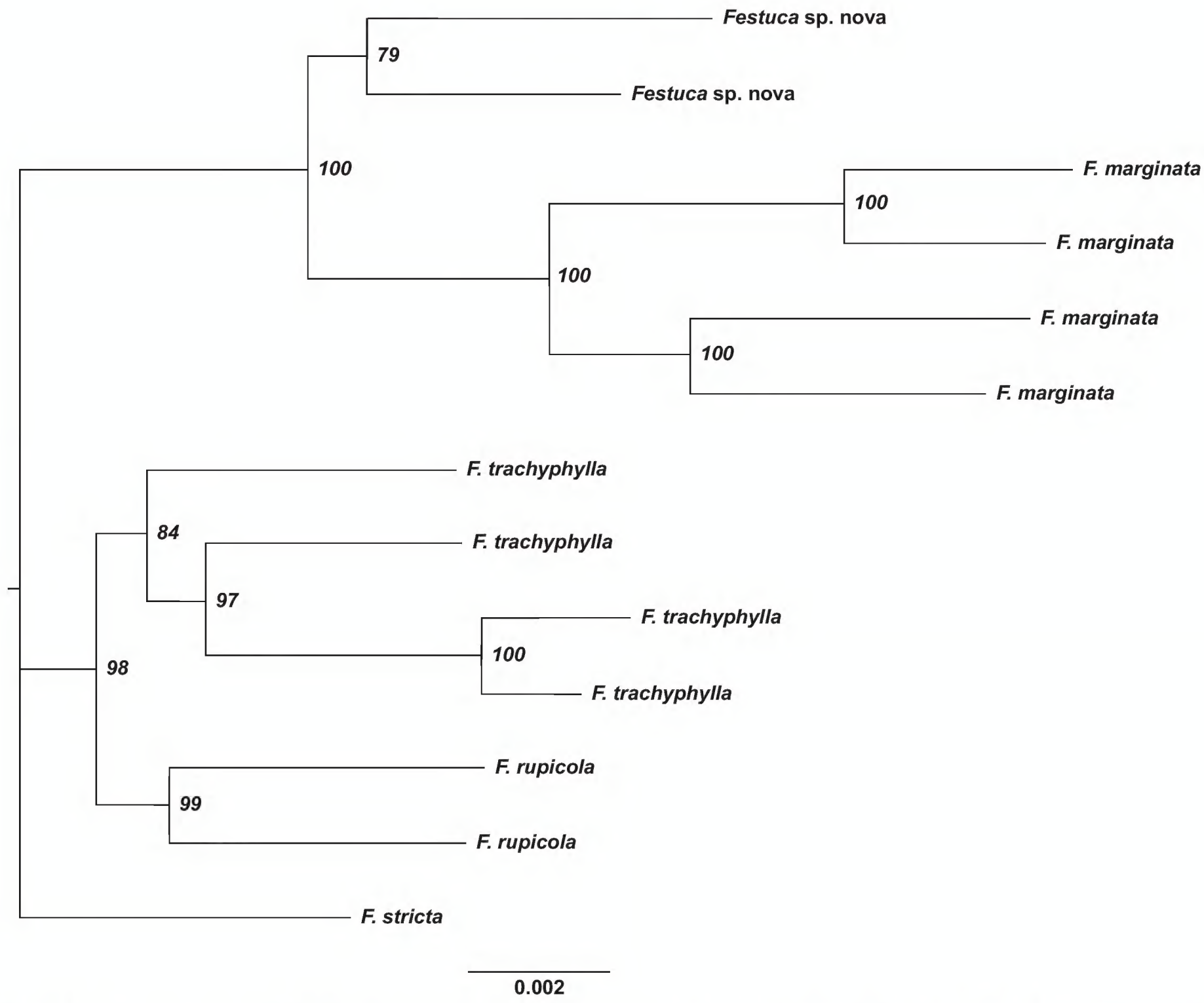

Figure 3. Phylogenetic tree of thirteen *Festuca* individuals, constructed using RAxML based on 2,713 loci and maximum likelihood estimation. The numbers in the nodes represent bootstrap values (%) from 1,000 replicates.

Phylogenomic analysis

A total of 67,353 loci were initially identified, which were reduced to 2,713 after filtering. The primary cause of this reduction was the requirement for a minimum of seven out of 13 samples to contain data for a given locus. Following the first analysis, one sample (F14) with low sequencing coverage was excluded based on quality assessment, and the analyses were repeated without this sample. The number of retained loci per sample ranged from 1,526 to 2,096. The resulting best tree effectively resolved each species, with high bootstrap support for most nodes (Fig. 3).

Taxonomic treatment

Festuca silana **Ardenghi, Pallanza & Foggi, sp. nov**
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Typus. ITALY • Sila piccola, Zagarise (Catanzaro), Latteria, Lato W del sentiero, Pendici SW del M. Gariglione (WGS84: 39°07'29.4"N, 16°37'32.8"E), 1587 m, prateria meso-xerofila con roccia granitica affiorante, assieme a *Patzkea paniculata*, 06.07.2014, *N. Ardenghi & L. Bernardo*. (***holotype*:** PAV-150000!; ***isotypes*:** FI!, CLU!, W!, G!).

Italy • Sila grande, Spezzano della Sila (CS), Vaccarizzo, Sponda S del Lago Cecita (WGS84: 39°22'13.5"N, 16°30'40.1"E), 1150 m, prato arido con *Astragalus parnassi* subsp. *clabricus*, su sabbie granitiche con affioramenti rocciosi, 05.07.2023, *M. Pallanza & L. Bernardo*. (***paratypes*:** PAV-150002!, PAV-150003!, FI!, CLU!, W!, G!).

Italy • Sila grande, Casali del Manco (CS), Lago Ariamacina, Sponda N del lago (WGS84: 39°20'00.8"N, 16°32'39.0"E), 1327 m, prato arido con *Astragalus parnassi* subsp. *clabricus*, su sabbie granitiche, 05.07.2023, *M. Pallanza & L. Bernardo*. (***paratypes*:** FI!, CLU!, W!, G!).

Italy • Sila grande, San Giovanni in Fiore (CS), Carlomagno, A W della SP211 (WGS84: 39°16'58.1"N, 16°32'36.1"E), 1535 m, prato mesofilo a *Patzkea paniculata*, su substrato granitico con spesso strato di suolo, 05.07.2023, *M. Pallanza & L. Bernardo*. (***paratypes*:** PAV-150001!, FI!, CLU!, W!, G!).

Description. Perennial herb, caespitose to densely caespitose, occasionally pruinose. Tiller shoots intravaginal. Culms (32.0–) 44.7–57.2 (–69.5) cm long and (0.52–) 0.77–1.04 (–1.29) mm in diameter, erect cylindrical, canaliculated, smooth to the touch, with 1 (–2) visible nodes, dark brown to black in color, located in its lower third. Cauline leaves 1 (–2), (1.6–) 2.6–4.0 (–5.3) cm long, sheathing the culm for (5.8–) 8.0–10.3 (–12.8) cm. Basal leaf sheath glabrous, on extremely rare occasions with few sparse hairs, open down to the base, yellowish in color, more rarely veined in red. Ligula 0.5–0.8 mm long, membranaceous, truncate, fringed, with two auricles at the sides. Basal leaf blade (7–) 11.8–21.6 (–31.7) cm long and (0.75–) 0.94–1.06 (–1.36) mm in diameter, smooth, somewhat rigid, conduplicate, bright to dark green. Cross section outline in an open U-V shape. Subepidermal sclerenchyma organized in 3 main strands located at the margins and keel of the leaf blade, thickened and often decurrent or accompanied by secondary strands opposing vascular bundles, forming an interrupted or irregular complete ring. Vascular bundles 7(–9). Ribs 2 (–4). Abaxial surface of the leaf blade covered with a dense indumentum of (0.03–) 0.04–0.07 (–0.13) mm long hairs. Adaxial surface smooth, with small undulations between epidermal cells. Panicle (4–) 6.4–8.6 (–12.5) cm long, cylindrical to pyramidal during anthesis, dense, with 7–27 spikelets; branches 1–5, simple, antrorsely scabrid; nodes 7–12. Spikelets (6.24–) 7.33–8.13 (–8.87) long, laterally flattened, elliptic, green, with (3–) 4–6 (–8) fertile florets. Glumes 2, unequal, lanceolate, glabrous or rarely with few sparse hairs on the margins and apex. Lower glume (2.21–) 2.55–3.12 (–3.90) mm long, with a single nerve. Upper glume (2.46–) 3.68–4.31 (–5.12) mm long, 3-nerved. Lemma (3.82–) 4.81–5.26 (–5.83) mm long, lanceolate, glabrous or more rarely with few sparse hairs near the apex, terminating in an apical awn (1.23–) 1.83–2.52 (–3.30) mm long. Palea 4.53–5.71 mm long, lanceolate, bifid, with 2 finely dentated keels. Anthers 3, (1.72–) 2–2.44 (–2.93) mm long, yellow to orange in color. Ovary glabrous. Stygmas 2, with a feather-like shape. Lodicules 2, bilobed, 0.83–1.06 mm long. Caryopsis 2.42–3.85 mm long, brown to dark orange at maturity, adherent to the palea (Fig. 4). Somatic chromosomes 42 (2n = 6x).

Eponym. The new species is named after the Sila plateau where it typically occurs.

Diagnosis. Differt a *Festuca costei* (St.-Yves ex Litard.) Markgr.-Dann. crassiore sclerenchyma in laminarum margine et carina, plerumque 7

(non (7–) 9–11) fasciculis vascularibus, densioribus et longioribus trichomatibus in abaxiale superficie, minutis undulationibus inter epidermidis cellulas, 42 (non 28) chromosomatibus.

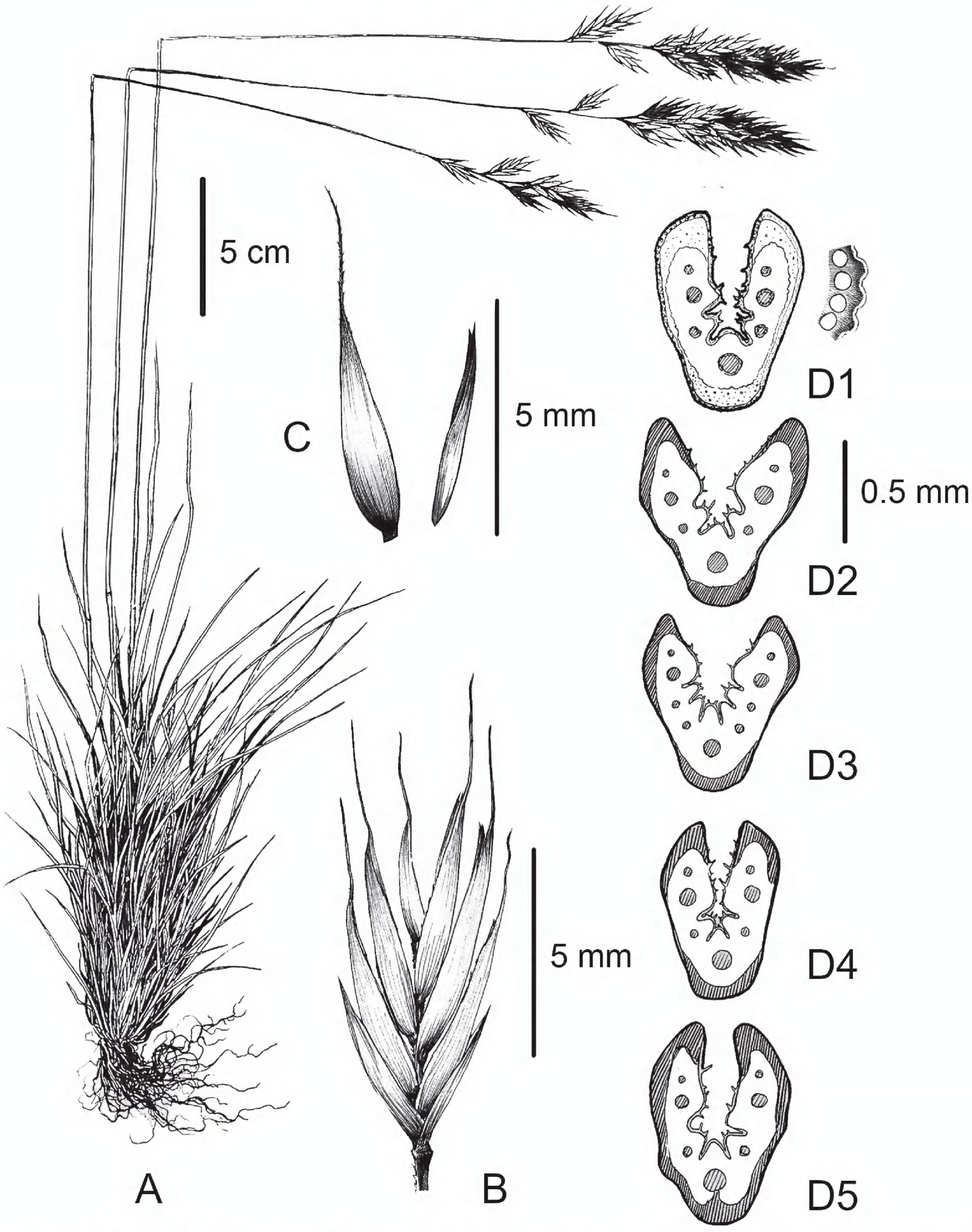

Figure 4. Illustration of *Festuca silana* Pallanza, Ardenghi & Foggi based on the specimens from the locus classicus **A** overall habitus and morphology of the species **B** detailed appearance of the spikelet **C** detailed view of lemma and palea **D1** leaf cross-section of the holotype **D2–D5** different leaves cross-sections showcasing intraspecific variability.

It differs from *Festuca costei* (St.-Yves ex Litard.) Markgr.-Dann. for the thicker sclerenchyma at margins and keel of the leaves, vascular bundles are rarely more than 7 (compared to the (7–) 9–11 in *F. costei*). Abaxial indumentum is more dense and with longer trichomes compared to *F. costei*. It also presents small undulations between epidermal cells. Somatic chromosomes 2n = 6x = 42 instead of 2n = 4x = 28 in *F. costei*.

Differt a *Festuca marginata* (Hack.) K.Richt. sclerenchyma plerumque decurrente vel completum anulum fingente potius quam in tribus discretis filis ordinato, fasciculis vascularibus raro plus quam 7 (non (7-) 9–11 ut solet in F. marginata), longioribus trichomatibus in densiore abaxiale superficie, longioribus aristis, undulationibus inter epidermidis cellulas, 42 chromosomatibus, non 14.

It differs from *Festuca marginata* (Hack.) K.Richt. in the sclerenchyma, usually decurrent or up to forming a complete ring instead of being organized in three discrete strands. Vascular bundles are rarely more than 7 (compared to the (7–) 9–11 in *F. marginata*). Abaxial indumentum is richer and with longer trichomes compared to *F. marginata*. Awns longer compared to *F. marginata*. Undulations are present in between epidermal cells. Somatic chromosomes 2n = 6x = 42 instead of 2n = 2x = 14 in *F. marginata*.

Differt a *Festuca trachyphylla* (Hack.) R.P.Murray sclerenchyma prope margines et carinam crassiore; foliis numquam scabris et colore viridi clariore (non glauco ut solet in *F. trachyphylla*); spiculis numquam pubescentibus; foliarum vaginis quam saepissime glabris (non pubescentibus ut solet in *F. trachyphylla*).

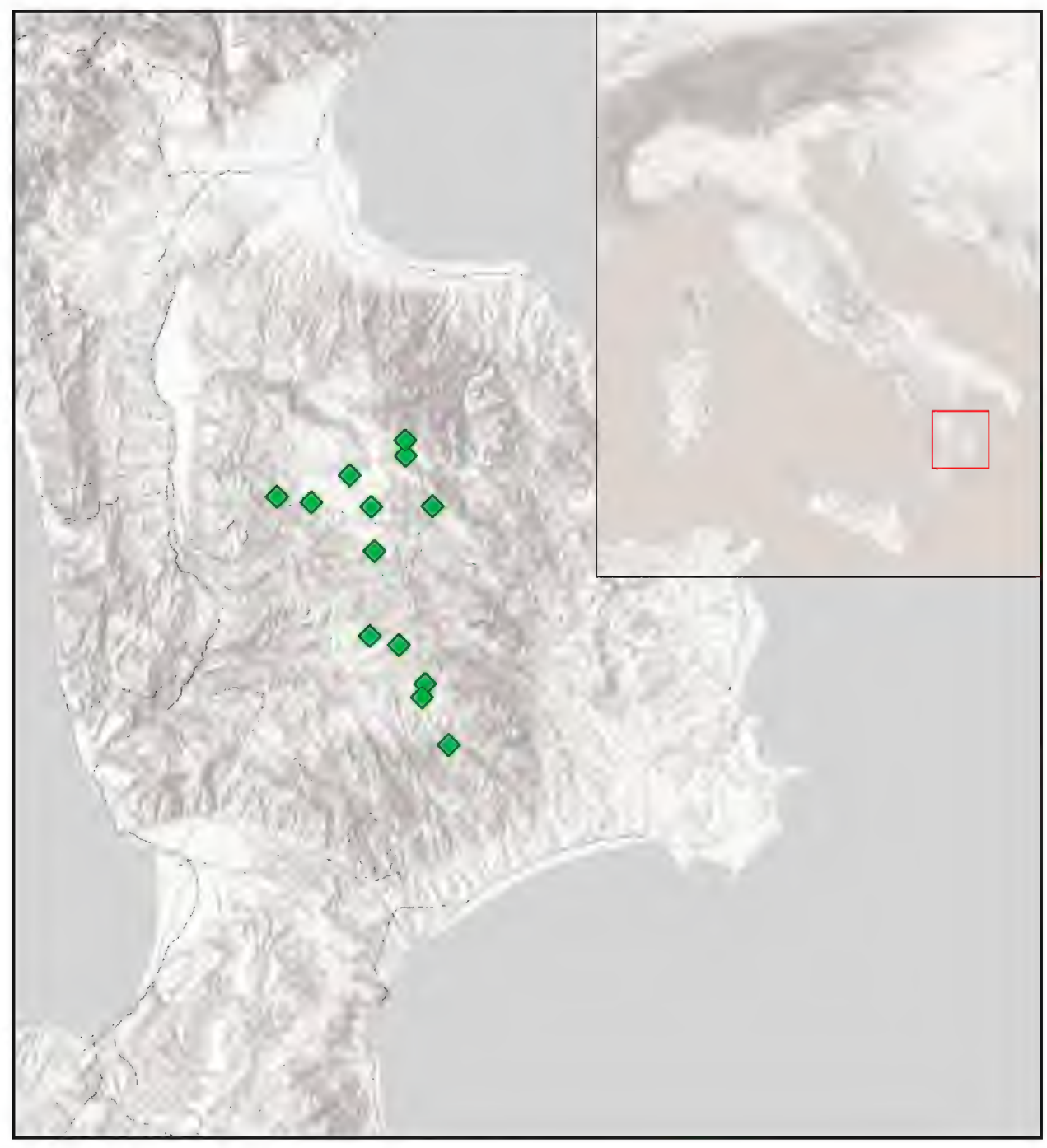

Figure 5. Map of the Sila and known stations of *F. silana* occurring within the plateau.

It differs from *Festuca trachyphylla* (Hack.) R.P.Murray in the sclerenchyma, thicker in the correspondence of margins and keel, the leaf blades never scabrid and of a brighter green color (opposed to the glaucus of *F. trachyphylla*). Spikelets are never pubescent. Leaf sheaths are glabrous except for extremely rare exceptions (opposed to the commonly pubescent in *F. trachyphylla*).

Distribution and ecology. The species is only known from the Sila highland, currently from 13 populations (Fig. 5). It mainly grows in grassland and pasture communities (*Anthemidetalia calabricae* Brullo, Scelsi & Spampinato, 2001) on granitic sands in xerophilic to mesophilic conditions at altitudes between 1100 m and 1600 m. *F. silana* typically occurs in grasslands of xerophile to mesophile conditions, along with other *Festuca* taxa such as *F. marginata* subsp. *marginata*, *F. trachyphylla*, *F. rubra* subsp. *commutata* and *F. cyrnea*.

Conservation status. Although the plant has a distribution limited to the Sila plateau, it is one of the dominant species in the grasslands that it inhabits and lacks any particular threat that could cause a decline in the population. Therefore, it had to be considered as Least Concern (LC) according to IUCN (2012).

Key to the studied species

1 Sclerenchyma strands decurrent sometimes with accessory strands or confluent in an irregular complete ring. Spikelets 7–8.5 mm. Awns usually > 2 mm long. Hexaploid plants **2**

– Sclerenchyma in three discrete strands, never decurrent. Spikelets 6–7 mm. Awns short, generally < 2 mm. Diploid plants. ..... ***F. marginata* subsp. *marginata***

2 Leaf scabrid, at least in the apicat/upper part. Spikelets and leaf sheaths generally pubescent. Plants glaucous to dark green. **3**

– Leaves completely glatt/smooth, never scabrid. Spikelets glabrous. Leaf sheaths usually glabrous, only very rarely with sparse hair. Plant bright/fresh green. ..... ***F. silana***

3 Tiller leaves with 5(rarely 7) vascular bundles. Sclerenchyma of regular thickness throughout its length. Plants of natural areas. **4**

– Tiller leaves with 7–9 (rarely 5) vacular bundles. Sclerenchyma irregularly thickened throughout its length. Plant typical of synantropic or disturbed habitats. ..... ***F. trachyphylla***

4 Sclerenchyma generally forming a thick continuous ring, more rarely partly interrupted. Leaves strongly scabrid. ..... ***F. stricta***

– Sclerenchyma in three decurrent strands at the margins and keel, more rarely with accessory strands opposed to the vascular bundles. Leaves moderately to weakly scabrid. ..... ***F. rupicola***

Discussion

Despite the superficial similarities with other taxa of the *Festuca marginata* group and *F. trachyphylla*, our findings fully support *F. silana* as a standalone species. Attempts to identify the samples of *F. silana* with current keys of the Italian flora led to *F. costei* due to the high importance of the sclerenchyma ring and the general appearance of plants resembling the *F. marginata* group. However, many morphological characters neglected by the keys

(such as the number of vascular bundles, the presence of undulations in-between epidermal cells, shorter arms of the leaf cross-section, etc.) easily emerge when looking at the specimens of the two taxa side by side. *F. costei* (= *F. arvernensis* Auquier, Kerguélen & Markgr.-Dann. subsp. *costei* (St.-Yves) Auquier & Kerguélen) has been described for the Massif Central in France and never reported for the Apennines. The separation between the two taxa was also confirmed by the ploidy inference via flow cytometry. *F. silana* is always hexaploid, thereby excluding any possibility of it being a particular morphotype of *F. costei*, which has been consistently reported as tetraploid (Auquier and Kerguélen 1977; Portal 1999; Šmarda et al. 2008). Additionally, although *F. costei* appears in the most recent checklists of the Italian vascular flora (Bartolucci et al. 2018, 2024), it has been reported for a single locality in the Maritime Alps (Piedmont, Italy; Foggi et al. 2017), but we could not trace any herbarium specimens supporting this data. Moreover, we visited the locality reported by Foggi et al. (2017) and could not find any individuals of this taxon here. Finally, biogeographical considerations were quite weak in supporting this determination and a disjunction between Massif Central – Southern Apennines is not very plausible. In light of these consideration, *F. costei* is not a component of the flora of Italy.

Despite the shared hexaploidy and similar leaf cross-sections' anatomy, fescues of the *F. stricta* group differ from *F. silana* in their commonly pubescent tiller leaves' sheaths and spikelets. Also, species of the *F. stricta* group commonly display some level of scabridity in the tiller leaf blades, which never occurs in *F. silana*. Finally, *F. silana* differs from *F. marginata* from the strong morphological distinction in leaf cross section anatomy, especially in sclerenchyma development and structure, as well as for the ploidy level (diploid vs hexaploid). The morphological differences can be attributed to the different ploidy levels, as it has been shown that an increase in ploidy level influences different morphological characters in fine-leaved fescues (Rewicz et al. 2018).

It is reasonable to think that *F. silana* may have originated from autopoliploidy of *F. marginata*, which is also present in the Sila plateau and sometimes even within the same localities. This hypothesis is supported by the ddRADseq sequencing results. It should be noted that the *F. marginata* group has recently been revised in Central-Southern Europe (Ardenghi et al. 2024) and all the previously described taxa have been reduced to two single diploid subspecies reflecting major differences in morphology and genome size between the populations in Greece (considered as *Festuca marginata* subsp. *heldreichii* (Hack.) Ardenghi & Foggi) and the remaining European populations (reported under *Festuca marginata* (Hack.) K. Richt. subsp. *marginata*). This newly discovered taxa from the Southern Apennines highlights the need for continued taxonomic research to broaden the understanding of plant diversity of critical groups such as *Festuca* in the Apennines.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: SO, MP, NMGA, PŠ, BF. Data curation: PŠ, LB, MP. Formal analysis: MP, PŠ, PŠ. Funding acquisition: GR, PŠ, SO. Investigation: PŠ, NMGA, LB, MP, BF. Methodology: LB, BF, NMGA, MP, PŠ, PŠ. Resources: BF, LB, PŠ. Supervision: GR, SO, BF. Writing - original draft: PŠ, SO, LB, BF, NMGA, GR, MP, PŠ. Writing - review and editing: PŠ, MP, GR, PŠ, NMGA, BF, LB, SO.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

References

Ardenghi NMG, Šmarda P, Calbi M, Coppi A, Lastrucci L, Lazzaro L, Mugnai M, Quercioli C, Rossi G, Foggi B (2024) Revision of the Festuca marginata "group" (Festuca sect. Festuca, Poaceae) in Southern Europe, with special reference to France, Italy and

Greece. Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology 158(5): 1192–1214. https://doi.org/10.1080/11263504.2024.2395870

Ardenghi NMG, Fiorini G, Rossi G, Foggi B (2016) Chromosome numbers and karyomorphology of Festuca circummediterranea Patzke group (Poaceae, Loliinae). Phytotaxa 263(3): 255–269. https://doi.org/10.11646/phytotaxa.263.3.6

Ardenghi NMG, Foggi B, Orsenigo S, Maggioni L, Cauzzi P, Rossi G (2017) Festuca and allied genera (Poaceae) as Crop Wild Relatives: Checklists and Red Lists are urgently required. Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology 151(1): 6–8. https://doi.org/10.1080/11263504.2016.1165753

Arndt S (2008) Novelties in the Festuca valesiaca group (Poaceae) from the central Alps. Plant Systematics and Evolution 271(3–4): 129–142. https://doi.org/10.1007/s00606-007-0588-0

Auquier P, Kerguélen M (1977) Un groupe embrouillé de Festuca (Poaceae): Les taxons désignés par l'épithète "glauca" en Europe occidentale et dans les régions voisines. Lejeunia 89: 1–82.

Bartolucci F, Peruzzi L, Galasso G, Albano A, Alessandrini A, Ardenghi NMG, Astuti G, Bacchetta G, Ballelli S, Banfi E, Barberis G, Bernardo L, Bouvet D, Bovio M, Cecchi L, Di Pietro R, Domina G, Fascetti S, Fenu G, Festi F, Foggi B, Gallo L, Gottschlich G, Gubellini L, Iamonico D, Iberite M, Jiménez-Mejías P, Lattanzi E, Marchetti D, Martinetto E, Masin RR, Medagli P, Passalacqua NG, Peccenini S, Pennesi R, Pierini B, Poldini L, Prosser F, Raimondo FM, Roma-Marzio F, Rosati L, Santangelo A, Scoppola A, Scortegagna S, Selvaggi A, Selvi F, Soldano A, Stinca A, Wagensommer RP, Wilhalm T, Conti F (2018) An updated checklist of the vascular flora native to Italy. Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology 152(2): 179–303. https://doi.org/10.1080/11263504.2017.1419996

Bartolucci F, Peruzzi L, Galasso G, Alessandrini A, Ardenghi NMG, Bacchetta G, Banfi E, Barberis G, Bernardo L, Bouvet D, Bovio M, Calvia G, Castello M, Cecchi L, Del Guacchio E, Domina G, Fascetti S, Gallo L, Gottschlich G, Guarino R, Gubellini L, Hofmann N, Iberite M, Jiménez-Mejías P, Longo D, Marchetti D, Martini F, Masin RR, Medagli P, Peccenini S, Prosser F, Roma-Marzio F, Rosati L, Santangelo A, Scoppola A, Selvaggi A, Selvi F, Soldano A, Stinca A, Wagensomme RP, Wilhalm T, Conti F (2024) A second update to the checklist of the vascular flora native to Italy. Plant Biosystems - An International Journal Dealing with All Aspects of Plant Biology 158(2): 219–296. https://doi.org/10.1080/11263504.2024.2320126

Bednarska I, Cwener A, Šmarda P (2024) Festuca makutrensis (Poaceae) in the flora of the Eastern Europe. Plant Systematics and Evolution 310(5): 33. https://doi.org/10.1007/s00606-024-01913-2

Boeuf R, Hardion L, Šmarda P, Lazare JJ, Thébaud G, Gregor T, Portal R, Höcker R, Mazas SR, Besnard G, Holveck P (2022) Des Carpates aux Pyrénées, centrées sur l'Alsace et les Vosges, analyses génétiques de quelques fétuques à feuilles fines (Festuca sect. Festuca) estimées d'intérêt régional à européen: Considérations taxinomiques et syntaxinomiques. Botanique 8: 37–133.

Brullo S, Scelsi F, Spampinato G (2001) La vegetazione dell'Aspromonte. Studio fitosociologico. Laruffa editore, Reggio Calabria.

Eaton DA, Overcast I (2020) ipyrad: Interactive assembly and analysis of RADseq datasets. Bioinformatics 36(8): 2592–2594. https://doi.org/10.1093/bioinformatics/btz966

Ellis RP (1976) A procedure for standardizing comparative leaf anatomy in the Poaceae. I. The leaf blade as viewed in transverse section. Bothalia 12(1): 65–109. https://doi.org/10.4102/abc.v12i1.1382

Foggi B, Tison JM (2014) Festuca L. In: Tison JM, de Foucault B (Eds) Flora Gallica. Flore de France. Biotope Éditions, Mèze, 236–248.

Foggi B, Rossi G, Signorini MA (1999) The Festuca violacea aggregate in the Alps and Apennines (central southern Europe). Canadian Journal of Botany 77(7): 989–1013. https://doi.org/10.1139/b99-054

Foggi B, Parolo G, Šmarda P, Coppi A, Lastrucci L, Lakušic D, Eastwood R, Rossi G (2012) Revision of the Festuca alpina group (Festuca section Festuca, Poaceae) in Europe. Botanical Journal of the Linnean Society 170(4): 618–639. https://doi.org/10.1111/j.1095-8339.2012.01313.x

Foggi B, Rossi G, Ardenghi NMG, Parolo G (2017) Gen. Festuca. In: Pignatti S (Ed.) Flora d'Italia 1. Edagricole, Milano, 550–582.

Govaerts R [Ed.] (2024) WCVP: World Checklist of Vascular Plants. Facilitated by the Royal Botanic Gardens, Kew. https://doi.org/10.48580/dglq4-4nz

Gower JC (1971) A general coefficient of similarity and some of its properties. Biometrics 27(4): 623–637. https://doi.org/10.2307/2528823

Gudkova PD, Kriuchkova EA, Shmakov AI, Nobis M (2023) Preliminary checklist of the genus *Festuca* L. (Loliinae, Pooideae, Poaceae) in the Altai Mountains with outlines for further studies. PhytoKeys 234: 229–274. https://doi.org/10.3897/phytokeys.234.105385

Hackel E (1882) Monographia Festucarum Europaearum. Theodor Fischer, Kassel. https://doi.org/10.5962/bhl.title.15610

IUCN (2012) IUCN Red List categories and criteria: version 3.1, 2nd edn. IUCN Species Survival Commission, Gland, Switzerland and Cambridge, UK.

Kellogg EA (2015) Flowering Plants. Monocots. Poaceae. In: Kubitzki K (Ed.) The Families and Genera of Vascular Plants 13. Springer. https://doi.org/10.1007/978-3-319-15332-2

Klecka WR 1980 Discriminant analysis (No. 19) Sage University Paper Series on Quantitative Applications in the Social Sciences, 07–019.

Kriuchkova EA, Baiakhmetov E, Nobis M, Gudkova PD (2023) First insight into the phylogeny of fine-leaved Festuca in the Altai Mountain Country based on genome-wide genotyping. Ecology and Evolution 13(4): e9943. https://doi.org/10.1002/ece3.9943

Krzanowski WJ (1990) Principles of multivariate analysis. Clarendon Press, Oxford.

Martínez-Sagarra G, Castro S, Mota L, Loureiro J, Devesa JA (2021) Genome size, chromosome number and morphological data reveal unexpected infraspecific variability in Festuca (Poaceae). Genes 12(6): 906. https://doi.org/10.3390/genes12060906

Mucko M, Lakušić D, Frajman B, Bogdanović S, Doboš M, Ljubičić I, Kuzmanović N, Rešetnik I (2024) Diversification of fescues (Festuca sect. Eskia, Poaceae), a key-component of southern European mountainous grasslands. Taxon 73(6): 1370–1390. https://doi.org/10.1002/tax.13278

Ortúñez E, Cano-Ruiz J (2013) Epidermal micromorphology of the genus Festuca L. subgenus Festuca (Poaceae). Plant Systematics and Evolution 299(8): 1471–1483. https://doi.org/10.1007/s00606-013-0809-7

Otto F (1990) DAPI staining of fixed cells for high-resolution flow cytometry of nuclear DNA. In: Crissman HA, Darzynkiewicz Z (Eds) Methods in Cell Biology 33: 105–110. https://doi.org/10.1016/S0091-679X(08)60516-6

Portal R (1999) Festuca de France. Vals-près-Le-Puy.

Posit team (2024) RStudio: Integrated Development Environment for R. Posit Software, PBC, Boston, MA. http://www.posit.co/

R Core Team (2024) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Rambaut A (2015) FigTree: Tree figure drawing tool. http://tree.bio.ed.ac.uk/software/figtree/

Rewicz A, Tomczyk PP, Kiedrzyński M, Zielińska KM, Jędrzejczyk I, Rewers M, Kiedrzyńska E, Rewicz T (2018) Morphometric traits in the fine-leaved fescues depend on ploidy level: The case of Festuca amethystina L. PeerJ 6(2): 5576. https://doi.org/10.7717/peerj.5576

Roleček J, Dřevojan P, Šmarda P (2019) First record of Festuca amethystina L. from the Transylvanian Basin (Romania). Contributii Botanice 54: 91–97. https://doi.org/10.24193/Contrib.Bot.54.6

Saint-Yves A (1913) Les Festuca de la section Eu-Festuca et leurs variations dans les Alpes-Maritimes. Annuaire Conservatoiure et Jardin Botanique de la Ville de Geneve 17: 1–218. https://doi.org/10.5962/bhl.title.15432

Sarfatti G (1959) Prodromo della Flora della Sila (Calabria). Webbia 15(1): 169–248. https://doi.org/10.1080/00837792.1959.10669697

Šlenker M, Koutecký P, Marhold K (2022) MorphoTools2: An R package for multivariate morphometric analysis. Bioinformatics 38(10): 2954–2955. https://doi.org/10.1093/bioinformatics/btac173

Šmarda P, Kočí K (2003) Chromosome number variability in Central European members of the Festuca ovina and F. pallens groups (sect. Festuca). Folia Geobotanica 38(1): 65–95. https://doi.org/10.1007/BF02803128

Šmarda P, Šmerda J, Knoll A, Bureš P, Danihelka A (2007) Revision of central European taxa of Festuca ser. Psammophilae Pawlus: Morphometrical, karyological and AFLP analysis. Plant Systematics and Evolution 266(3–4): 197–232. https://doi.org/10.1007/s00606-007-0532-3

Šmarda P, Bureš P, Horová L, Foggi B, Rossi G (2008) Genome Size and GC Content Evolution of Festuca: Ancestral Expansion and Subsequent Reduction. Annals of Botany 101(3): 421–433. https://doi.org/10.1093/aob/mcm307

Sochor M, Šarhanová P, Duchoslav M, Konečná M, Hroneš M, Trávníček B (2024) Plant kleptomaniacs: Geographical genetic patterns in the amphi-apomictic Rubus ser. Glandulosi (Rosaceae) reveal complex reticulate evolution of Eurasian brambles. Annals of Botany 134(1): 163–178. https://doi.org/10.1093/aob/mcae050

Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30(9): 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Thiers BM (2024) [updated continuously] Index Herbariorum. https://sweetgum.nybg.org/science/ih/

Wilkinson MJ, Stace CA (1991) A new taxonomic treatment of the Festuca ovina L. aggregate (Poaceae) in the British Isles. Botanical Journal of the Linnean Society 106(4): 347–397. https://doi.org/10.1111/j.1095-8339.1991.tb02298.x

Supplementary material 1

Occurrences, studied specimens, protocols

Authors: Mattia Pallanza, Orsenigo Simone, Petr Šmarda, Petra Šarhanová

Data type: xlsx

Explanation note: Excel file with a guide sheet describing the content of the different other sheets. A List of herbaria specimens studied as an exploratory part of the research, datasets for the PCoA and DA, Protocols and information on the samples used in the DNA extraction and ddRAD sequencing are available.

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